REMARKS

The Applicants thank the Examiner for her careful analysis of the application and claims in this case. Claims 1, 4, 12, 14, 16 and 18-23 are pending in this case. All claims stand rejected.

The Examiner has acknowledged the priority claim of the application, but states that the provisional application does not provide adequate support to the application as required by 35 U.S.C. 112. It is noted that the Examiner has rejected all of the claims of the instant utility application under the same statute. The Applicants submit that the claims of the instant application are supported by both the provisional patent application and the specification of the instant application as discussed in detail below. Therefore, the priority claim is valid.

The Examiner has stated that no explanation of the non-English reference cited in the Information Disclosure Statement was provided. The Applicants have enclosed herewith a copy of the abstract of the reference in English from the EPO website and a copy of the PCT application which is stated to be equivalent to the French application. The Applicants state that the omission of the English version of the reference was inadvertent.

The Applicants thank the Examiner for her reconsideration of the restriction requirement and the acceptance of wild-type, mutated and truncated forms of phospholamban (PLB) as a single group.

The Examiner has rejected all of the claims in the case under 35 U.S.C. §112, paragraphs 1 and 2. The Examiner states that the claims are rejected under paragraph 1 for containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make or use the invention. The Examiner states that the specification does not teach how one would deliver the claimed peptides to myocytes and subsequently to the sarcoplasmic reticulum (SR). The Examiner states that a peptide the size of phospholamban would not readily enter the cell. PLB is a pentamer that can be dissociated both by heat and under certain buffer conditions that

are not incompatible with drug delivery. These conditions include specific salt concentrations and the absence of detergents. Such properties of phospholamban are well known to those skilled in the art. The Applicants submit that a phospholamban peptide attached to a penetratin peptide could readily be transported across a plasma membrane. In Derossi et al (*J. Biol. Chem*, **269**:10444-50. 1994, copy enclosed), the translocation of proteins of a size comparable to a PLB-penetratin peptide fusion is taught at a relatively low concentration of peptide. On page 10448, second column, first incomplete paragraph, it states:

> In most studies involving pAntp [the antentapedia homeodomain], we found strong accumulation of the homeodomain in the cell nuclei even when the 60-amino acid long polypeptide was added to the cells at an extracellular concentration of 5×10^{-8}M.

Translocation of a penetratin peptide across a membrane is believed to involve multimerization of the homeodomain; therefore, the effective size of the transported protein is larger than the penetratin peptide-PLB fusion. Further studies on penetratin peptides have demonstrated that the translocation is receptor and energy independent. Derossi et al. stated that based on the large amount of characterization and understanding of the penetratin peptides, that the data "open the way to the molecular design of cellular vectors (*J. Biol. Chem.*, **271**:18188-93. 1996, copy enclosed).

The Applicants call the Examiner's attention to the Franklel et al. (US Patent 5,652,122) cited in the Information Disclosure Statement filed by the Applicants. Data are provided to demonstrating that a peptide of 123 aa in length, fused to a penetratin peptide from TAT, is transported in sufficient quantities across a membrane to have a physiological effect (see Figure 13, Table II, column 40, lines 21-25). This is substantially larger than the PLB-penetratin peptide of the instant invention. The Applicants submit that the mechanism by which the peptide enters the cell need not be known for the claim to be enabled. Therefore, the Applicants submit that at the time of the application, those skilled in the art were knowledgeable about the use of penetratin peptides and would have been

able to design peptides that would be delivered into the cell.

The Examiner states that once the peptides entered the cell, the phospholamban would not insert into the SR. Typically, transmembrane proteins are inserted into the membrane co-translationally as directed by a signal sequence or present in the protein. Phospholamban is not inserted into the membrane by this mechanism. Phospholamban does not contain a signal sequence (Fujii et al., *J. Clin, Invest.*, **79**:301-4. 1987) and is translocated post-translationally through the membrane in a manner that is not fully understood. However, translocation occurs. The Applicants submit that the PLB-penetratin peptide would be inserted into the membrane by the same mechanism.

The Examiner states that specific delivery to cardiac myocytes would be difficult to accomplish as cardiac myocytes are only a small portion of the myocytes in the body. The Applicants submit that a number of devices for delivery of compounds to the pericardium were well known at the time of filing of the instant application. The Applicants have enclosed a few representative patents (Igo, US Patents 5,634,895 and 5,827,216 issued June 3, 1997 and October 28, 1998 respectively; and Grabek, US Patent 5,931,810 issued August 3, 1999) to demonstrate methods and apparatuses for delivery of material to the heart at the time of the application. By delivery of the protein to the pericardium, the cardiac myocytes would be specifically targeted.

Example 5 provides preliminary suggestive data regarding the efficacy of the PLB-penetratin peptides in altering contractility in cardiomyocytes as compared to control cardiomyocytes. This demonstrates that the peptide enters cells in sufficient quantities to have an effect. Delivery of peptides to the pericardium allows the cells to be in contact with the peptide for an extended period of time, allowing uptake of the peptide. Therefore, it is expected that sufficient peptide would be taken up by the cells and a modulation in contractility would be observed. Thus, the rejection of the claims under 35 U.S.C. §112, paragraph 1 is traversed.

The Examiner has rejected all of the claims under 35 U.S.C. §112, paragraph 2 for

failing to point out and distinctly claim the invention. The Examiner states that the specification does not teach how one would induce phospholamban deficiency. The claims have been amended to recite that the phospholamban is a dominant negative phospholamban and that it is delivered to cardiac tissue. The concept of a dominant negative factor is well known to those skilled in the art. A dominant negative factor inactivates the endogenous, wild-type factor frequently by irreversibly binding to an effector molecule preventing it from functioning.

Dominant negative mutants of phospholamban are discussed broadly in the specification on page 4, lines 17-19 and data demonstrating the inhibitory effect of one of the dominant negative mutants are shown in Table 2 and Figures 7 (p 19, ln 4-8) and 8 (p 19, ln 24-28). Inactivation of the endogenous phospholamban prevents its inhibition of SERCA2. In the case of the K3E/R14E double mutant of phospholamban, it is suggested that highly stable multimers containing more than five phospholamban monomers are formed based on the banding pattern observed in Western blots (page 18, line 23-28); however, it should be noted that the mechanism of action of a dominant negative phospholamban is not a limitation of the instant invention. By forming complexes with endogenous phospholamban that are more stable than complexes containing endogenous phospholamban alone, the dominant negative protein causes an effective deficiency. This deficiency increases cardiac contractility in a manner similar to reducing phospholamban levels using an antisense RNA or construct. Therefore, an effective knockout is created by the dominant negative protein mimicking the situation in the double knockout mouse.

The Applicants submit that determining if an specific mutant form of phospholamban mutant could act in a dominant negative fashion is well within the ability of those skilled in the art. The Applicants submit that a variety of phospholamban mutants are available to those skilled in the art (see Toyofuko et al., 1994, cited in the IDS). Upon publication in most peer review journals, an individual is required to share non-limited reagents (e.g. plasmid DNA) upon request. Based on the teachings of the application, one skilled in the

art would be able to readily determine forms of phospholamban that had the desired effect. Such assays would not constitute undue experimentation.

The Examiner has stated that one skilled in the art would not know how to prepare the peptides of the instant invention or deliver them to the heart. Generation of proteins that will be taken up by cardiomyocytes and methods of delivery are discussed above and references are provided. The Applicants submit that the rejections under 35 U.S.C. §112, paragraph 2 are traversed.

FEES

It is believed that no fee is due with this response. However, if a fee is due, the Commissioner is hereby entitled to charge Deposit Account 02-4070 referencing case number 6627-PA9025.

CONCLUSIONS

In view of the forgoing arguments and amendments, the Applicants submit that the application is now in proper form for allowance. If any outstanding issues remain, the Examiner is invited to call the agent for Applicant collect at the number listed below.

Respectfully submitted,

Dated: September 3, 2003

By: _____

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Docket No.: 6627-PA9025

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